

AMENDMENTS TO THE SPECIFICATION

[0018] Northern analysis of transgenic tobacco plants of the line 35S-DOG. Whole RNA was isolated from tobacco leaves, separated by gel electrophoresis and transferred onto a nylon membrane. Hybridization was performed with a radioactively labeled coding region of the DOG<sup>R1</sup> gene. 20 ~~mg~~ <sup>μg</sup> RNA were applied per lane. Lanes 1-9, independently transformed plants of the line 35S-DOG; lane 10, untransformed control.

[0041] Also, the introduction of point mutations at positions is conceivable in which a change in the amino acid sequence may have an influence on, e.g., the enzyme activity or the regulation of the enzyme. In this way, e.g., mutants with a modified ~~K<sub>m</sub>~~ K<sub>m</sub> value may be produced, or mutants which are no longer subject to the regulatory mechanisms by allosteric regulation or covalent modification usually occurring in cells.

[0082] Plant whole RNA was isolated according to the method by Logemann (1987, Analytical Biochem. 163, 16) and separated on formaldehyde agarose gels (Lehrach (1977) Biochem. 16, 4743). A capillary transfer on nylon membranes (Gene Screen, NEN) was carried out in 20 x SSC (1.5 M NaCl, 150 mM sodium citrate) overnight. After pre-hybridization for two hours in hybridization buffer (500 mM sodium phosphate (pH 7.2), 7% SDS, 1% bovine serum albumin, 200  $\mu$ g herring sperm DNA, 1 mM EDTA), the hybridization was carried out at 55°C for 16 hours with the radioactively labeled DOG<sup>R1</sup> probe. As probe the DOG<sup>R1</sup> coding region was isolated from plasmid pGEMT and radioactively labeled in the presence of  ~~$\alpha$ -<sup>32</sup>P-dCTP~~  $\alpha$ -<sup>32</sup>P-dCTP

using a High Prime Kit (Boehringer). Then, the filters were washed under the following conditions: 20 minutes at 55°C in 6 x SSC, 0.1% SDS and 20 minutes at 55°C in 4 x SSC, 0.1% SDS.

[0085] The DOG<sup>R</sup>1 coding region was cloned by Polymerase Chain Reaction (PCR). The matrix material used was genomic yeast DNA which was isolated from *Saccharomyces cerevisiae* strain S288C according to standard protocol. Amplification was carried out using the specific primers DOG<sup>R</sup>1-1 (5'-ATGGATCCCCATGGCAGAATTTTCAGCTGATCTATG-3'; SEQ ID NO:3) and DOG<sup>R</sup>1-2 (5' ATGTCGACTACTCAGGCCCTTGTCAAAGGGTTG-3'; SEQ ID NO:4), which were derived from a published sequence (Sanz (1994) Yeast 10, 1195-1202). Primer 1 includes bases 1 to 26 and primer 2 bases 720 to 741 of the coding region of the DOG<sup>R</sup>1 gene. For the cloning of the amplified DNA into plant expression vectors the primers additionally carry the following restriction sites: primer 1, BamHI and NcoI; primer 2, SalI. The cloning strategy is depicted in Figure 3. The PCR reaction mixture (50µl) contained chromosomal yeast DNA (1µg), primers 1 and 2 (1µg each), 10 mM Tris-HCl (pH 8.8 at 25°C), 3.5 mM ~~MgCl<sub>2</sub>~~ MgCl<sub>2</sub>, 50 mM KCl, 0.1% Triton X-100, 200 µM dNTPs (dATP, dCTP, dGTP, dTTP) and 2 units PrimeZyme DNA Polymerase (Biometra). Before the polymerase was added the mixture was heated to 94°C for 10 minutes. The polymerization steps (60 cycles) were carried out in an automated "Thermocycler" (Perkin Elmer) according to the following program: denaturation at 94°C (1 minute), annealing of the primers at 40°C (1 minute), polymerase reaction at 72°C (1 minute). The fragment obtained was cloned into the vector pGEMT (Promega Corp., 2800 Woods Hollow Road, Madison, WI 53711-5399, USA)

and the plasmid pGEMT- DOG<sup>R</sup>1 was obtained. The identity of the amplified DNA was verified by sequence analysis.